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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER
KAM, CHIH MIN

ART UNIT	PAPER NUMBER
1653	17

DATE MAILED: 05/28/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Application No.	Applicant(s)
09/866,538	TSIEN ET AL.
Examiner	**Art Unit**
Chih-Min Kam	1653

Office Action Summary

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 March 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *88-153* is/are pending in the application.

4a) Of the above claim(s) *90-92,100,101,111-127,129-133,135-137 and 139-153* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *88,89,93-99,102-110,128,134 and 138* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a)☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

DETAILED ACTION

Election/Restrictions

1. Applicant's election with traverse of Group I, claims 88-110, 128, 134 and 138, Aequorea GFP related protein, an enhanced CFP (SEQ ID NO:6), and an A206K mutation of SEQ ID NO:6 in Paper No. 16 is acknowledged. The traversal is on the ground(s) that all claims share common structural, functional, and utility elements, e.g., the claims include at least a polynucleotide that encodes a non-oligomerizing tandem fluorescent protein; the inventions have not required a separate status in the art and do not require different searches; additional restriction of the fluorescent protein species of Inventions I and II is inappropriate and such proposed restriction would appear more consistent with election of species practice; and restriction among Inventions I-IV and the further restriction between proteins place a serious burden on the Applicant. This is not found persuasive because the products of Inventions I-IV are directed to structurally and functionally different nucleotides, e.g., nucleotides of Invention I are drawn to a polynucleotide encoding a non-oligomerizing tandem fluorescent protein, which is a homopolymer having a property of reducing oligomerizing; while nucleotides of Invention II are drawn to a polynucleotide encoding a tandem non-oligomerizing fluorescent protein, which is a heteropolymer and can be used for fluorescence resonance energy transfer; nucleotides of Invention III are drawn to a nucleic acid comprising a polynucleotide encoding a non-oligomerizing tandem fluorescent protein and operatively linked to at least a second polynulceotide; nucleotides of Invention IV are drawn to a nucleic acid comprising a polynucleotide encoding a tandem non-oligomerizing fluorescent protein and operatively linked to at least a second polynucleotide, therefore the nucleotides of Inventions I-IV are patentably

distinct. Furthermore, various fluorescent proteins such as GFP and RFP having different amino acid sequences exhibit different spectral properties, thus these proteins are patentably distinct. Regarding search burden, coexamination of each of the additional groups and sequences would require search of classes and sequences not necessary for Group I. For example, if Group III were included, it would require additional search of class 424, subclass 192.1; if DsRed were included, it would require additional search of SEQ ID NO:12. Therefore, coexamination of each of these inventions would require a serious burden of search. Upon reconsideration, unmodified Aequorea GFP (SEQ ID NO:2), SEQ ID NO:10, and A206K, L221K and F223R mutations of SEQ ID NOs:6 and 10 will be included for examination. Claims 111-127, 129-133, 135-137 and 139-153 in Groups II-IV, and claims 90-92 and 100-101 in Group I, are directed to non-elected inventions, thus withdrawn from consideration. Therefore, claims 88, 89, 93-99, 102-110, 128, 134 and 138 and SEQ ID NOs: 2, 6 and 10 are examined.

The requirement is still deemed proper and is therefore made FINAL.

Claim Objections

2. Claims 89, 93, 94, 95 and 98 are objected to because the claims contain recitation of non-elected sequences.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

3. Claims 88, 89, 93-99, 102-110, 128, 134 and 138 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. The claim is drawn to a polynucleotide sequence encoding a non-oligomerizing tandem fluorescent protein. As

written, the claim does not explicitly indicate the hand of man. Insertion of "isolated" in connection with a polynucleotide is suggested. See MPEP § 2105.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 88, 89, 93-99, 102-110, 128, 134 and 138 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a polynucleotide encoding a non-oligomerizing tandem fluorescent protein, wherein the protein comprises a first monomer of a fluorescent protein operatively linked to at least second monomer of the fluorescent protein, wherein the sequence of the monomer is defined (e.g., the A206K, L221K or F223R mutant of SEQ ID NO:6 or 10), and wherein the propensity of the tandem fluorescent protein is reduced or inhibited; a vector or a host cell comprising the polynucleotide; a kit comprising the polynucleotide; or, a polynucleotide encoding a fusion protein, wherein the fusion protein comprises the non-oligomerizing tandem fluorescent protein operatively linked to at least one polypeptide of interest, wherein the polypeptide of interest is defined (e.g., polyHis tag), does not reasonably provide enablement for a polynucleotide encoding a non-oligomerizing tandem fluorescent protein, wherein the protein comprises a first monomer of a fluorescent protein operatively linked to at least second monomer of the fluorescent protein, wherein the sequence of the monomer is not defined, and wherein the propensity of the tandem fluorescent protein is reduced or inhibited; a vector or a host cell comprising the polynucleotide; a kit comprising the polynucleotide; or, a polynucleotide encoding a fusion protein, wherein the fusion protein

comprises the non-oligomerizing tandem fluorescent protein operatively linked to at least one polypeptide of interest, wherein the polypeptide of interest is not defined. The specification does not enable a person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Claims 88, 89, 93-99, 102-110, 128, 134 and 138 encompass a polynucleotide encoding a non-oligomerizing tandem fluorescent protein, wherein the protein comprises at least two monomers of a fluorescent protein, wherein the propensity of the tandem fluorescent protein is reduced or inhibited (claims 88, 89, 93-99 and 102); a vector (claim 128) or a host cell (claims 134) comprising the polynucleotide; a kit comprising the polynucleotide (claim 138); or a polynucleotide encoding a fusion protein, wherein the fusion protein comprises the non-oligomerizing tandem fluorescent protein operatively linked to at least one polypeptide of interest (claims 103-110). The specification however, only discloses cursory conclusions without data supporting the findings, which state that the invention relates to a polynucleotide encoding a non-oligomerizing tandem fluorescent protein, which includes at least two monomers of a fluorescent protein, wherein the propensity of the tandem fluorescent protein is reduced or inhibited as compared to a monomer of the fluorescent protein, and the fluorescent protein can be a GFP, a RFP, or a fluorescent protein related to a GFP or a RFP (pages 2-3, paragraph [0008]; page 4, paragraph [0014]). There are no indicia that the present application enables the full scope in view of a non-oligomerizing tandem fluorescent protein and a fusion protein containing the tandem fluorescent protein as discussed in the stated rejection. The present application provides no indicia and no teaching/guidance as to how the claims are enabled. The factors considered in determining whether undue experimentation is required, are summarized in In re Wands (858

F2d at 731,737, 8 USPQ2d at 1400,1404 (Fed. Cir.1988)). The factors most relevant to this rejection are the scope of the claims, the state of the prior art, the amount of direction or guidance presented, and the amount of experimentation necessary.

(1). The breath of the claims:

The breath of the claims is broad and encompasses unspecified variants regarding identities of non-oligomerizing fluorescent proteins (the monomer of fluorescent proteins) and of the polypeptide of interest in the fusion protein containing the non-oligomerizing tandem fluorescent protein, which are not adequately described or demonstrated in the specification.

(2). The presence of working examples:

The specification has shown the A206K, L221K and F223R mutants of SEQ ID NOs:6 and 10 are non-oligomerizing fluorescent protein (Example 1), where the replacement of the hydrophobic residues A206, L221 and F223 with residues containing positively charged side chains eliminate dimerization, while ECFP (SEQ ID NO:6) and EYFP-V68L/Q69K (SEQ ID NO:10) formed a dimer with high affinity of 113 μM; and the I125R mutant of DsRed reduced the propensity of DsRed to form tetramer, while DsRed I125K mutant was a mixture of dimer and tetramer (Example 3), and a tandem DsRed protein is formed by linking two DsRed monomers (Example 4). However, there are no other working examples indicating the claimed variants.

(3). The state of the prior art and relative skill of those in the art:

The specification (paragraph [0059]) and the prior art (e.g., Yang et al., Nature Biotechnology 14, 1246-1251 (1996)) indicate a head-to tail, side by side dimer is formed in the crystal of GFP and its variants, and a core of hydrophobic side chains such as A206, L221 and

F223 from each of the two monomers appear to be candidates for creating the contacts between monomers when GFP is in solution or expressed exogenously in cells. However, the general knowledge and level of the skill in the art do not supplement the omitted description, the specification needs to provide specific guidance on the identities of non-oligomerizing fluorescent proteins and a polypeptide of interest in the fusion protein containing non-oligomerizing tandem fluorescent protein to be considered enabling for variants.

(4). The amount of direction or guidance presented and the quantity of experimentation necessary:

The claims are directed to a polynucleotide encoding a non-oligomerizing tandem fluorescent protein comprising at least two monomers of a fluorescent protein, wherein the propensity of the tandem fluorescent protein is reduced or inhibited as compared to the monomer; a vector, a host cell, or a kit comprising the polynucleotide; or a polynucleotide encoding a fusion protein, wherein the fusion protein comprises the non-oligomerizing tandem fluorescent protein operatively linked to at least one polypeptide of interest. The specification indicates the specific A206K, L221K and F223R mutants of SEQ ID NOs:6 and 10 are non-oligomerizing fluorescent protein (Example 1), while ECFP (SEQ ID NO:6) and EYFP-V68L/Q69K (SEQ ID NO:10) formed a dimer with high affinity of 113 μM; the I125R mutant of DsRed reduced the propensity of DsRed to form tetramer, while DsRed I125K mutant was a mixture of dimer and tetramer (Example 3); and a tandem DsRed protein is formed by linking two DsRed monomers (Example 4). However, the specification has not identified other non-oligomerizing fluorescent proteins, nor has demonstrated the effects of reducing oligomerizing in the tandem fluorescent proteins. Moreover, there are no working examples indicating various

non-oligomerizing tandem fluorescent proteins except for a tandem DsRed protein. Furthermore, the specification has not demonstrated the making and use of a fusion protein containing a defined non-oligomerizing tandem fluorescent protein and a defined target polypeptide except for a polyHis tag. Since the specification fails to provide sufficient teachings on identities of various non-oligomerizing fluorescent proteins as the monomers of tandem fluorescent proteins, and the effects of reducing oligomerizing in these tandem fluorescent proteins, it is necessary to have additional guidance on the identities of the non-oligomerizing fluorescent proteins and to carry out further experimentation to assess the effects of reducing oligomerizing in these tandem fluorescent proteins.

(5). Predictability or unpredictability of the art:

The specification indicates fluorescent proteins such as ECFP (SEQ ID NO:6) and EYFP-V68L/Q69K (SEQ ID NO:10) formed a dimer with high affinity of 113 μM, while the A206K, L221K and F223R mutants of SEQ ID NOs:6 and 10 are identified as non-oligomerizing fluorescent proteins (Example 1); and the DsRed I125K mutant was a mixture of dimer and tetramer, while the I125R mutant of DsRed reduced the propensity of DsRed to form tetramer (Example 3), thus, it requires to identify a non-oligomerizing fluorescent protein for the claimed invention. Since the claims encompass numerous unidentified variants, the effects of reducing oligomerizing in the tandem fluorescent proteins are unpredictable.

(6). Nature of the Invention

The scope of the claims includes many structural variants, however the specification has not identified these variants nor indicated the effects of reducing oligomerizing in the tandem fluorescent proteins. Thus, the disclosure is not enabling for reasons discussed above.

In summary, the scope of the claim is broad, while the working example does not demonstrate the claimed variants, and the guidance and the teaching in the specification is limited, therefore, it is necessary to have additional guidance and to carry out further experimentation to assess the effect of reducing oligomerizing of the tandem fluorescent protein.

Conclusion

5. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Chih-Min Kam whose telephone number is (703) 308-9437. The examiner can normally be reached on 8.00-4:30, Mon-Fri.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on (703) 308-2923. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-0294 for regular communications and (703) 308-4227 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Chih-Min Kam, Ph. D. CMK
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May 26, 2003